

Alcohol drinking, ADH1B and ADH1C genotypes, and the risk of postmenopausal breast cancer by hormone receptor status: The Netherlands Cohort Study on Diet and Cancer

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Abstract

Alcohol has consistently been shown to increase breast cancer (BC) risk. This association may be modified by single nucleotide polymorphisms in alcohol dehydrogenase isoenzymes *ADH1B* and *ADH1C*. The Netherlands Cohort Study comprises 62 573 women, aged 55-69 years at baseline (1986). Follow-up for postmenopausal BC for 20.3 years was available. Genotyping of 6 tag SNPs in *ADH1B* and *ADH1C*, respectively, was performed on DNA from toenails. A case-cohort approach was used for analysis (complete data available for: $n_{\text{subcohort}} = 1301$; $n_{\text{cases}} = 1630$). Cox regression models for postmenopausal BC were applied to determine marginal effects of alcohol intake and SNPs using a dominant genetic model, as well as multiplicative interaction of the two. Results were also obtained for subtypes by estrogen (ER) and progesterone receptor (PR) status. Multiple testing was adjusted for by applying the false discovery rate (FDR). Alcohol intake (categorical) increased the risk of postmenopausal BC ($p_{\text{trend}} = 0.031$). Trends for ER and PR subgroups followed a similar pattern. Continuous modelling of alcohol resulted in a hazard rate ratio (HR) for overall postmenopausal BC of 1.09 (95% CI: 1.01 – 1.19) per 10g/d of alcohol. SNPs were not associated with BC risk. No effect modification of the alcohol-BC association by SNP genotype was seen after FDR-correction in overall BC and ER/PR subgroups. In conclusion, alcohol was shown to increase the risk of postmenopausal BC. This association was not significantly modified by common *ADH1B* and *ADH1C* SNPs, neither in overall BC nor in hormone receptor defined subtypes.

SUMMARY:

Drinking alcohol is associated with increased breast cancer risk. Common SNPs in *ADH1B* and *ADH1C* might modify this association. In the Netherlands Cohort Study, no such modification is seen, neither in overall disease nor in hormone receptor subtypes.

Introduction

Breast cancer (BC), being the most frequently diagnosed cancer in females as well as the second most common cause of cancer death in females worldwide, accounted for 1.7 million cases and 521 900 deaths in 2012[1]. Alcohol consumption has consistently been shown to be an important risk factor[2,3]. Meta-analyses of observational studies show increased risk estimates already for one drink per day with a linear dose response increase. The population attributable proportion of BC due to alcohol consumption ranges between two percent for the United States and five percent for Europe[4,5]. The precise mechanisms of alcohol-associated tumorigenesis in BC are under discussion[3,6,7]. Two effects are considered to be especially important, the carcinogenic metabolite acetaldehyde and the estrogen-enhancing effect of ethanol[6]. Enhanced estrogen levels result in an increased cumulative lifetime exposure, which is an important risk factor for BC[8]. Both effects are closely linked to alcohol metabolism. Reviews showed a positive association between alcohol and estrogen receptor (ER) positive as well as ER negative tumors, with effect estimates being larger in ER+ tumors[9,10]. Acetaldehyde is a mutagenic and carcinogenic compound that causes formation of DNA adducts and inhibits DNA repair mechanisms[11].

The first step in alcohol metabolism is the oxidation process that leads to acetaldehyde, catalyzed by the enzyme alcohol dehydrogenase (ADH). Subsequently acetaldehyde is oxidized to acetate by acetaldehyde dehydrogenase (ALDH)[12]. Both enzymes are subject to genetic variability by SNPs. Individuals who carry alleles that cause an accumulation of acetaldehyde usually consume less alcohol due to the unpleasant effects of acetaldehyde, such as flushing. Alleles of *ALDH* that impact alcohol tolerance are widely absent in Caucasian populations. However, the ADH gene cluster consists of seven isoenzymes. *ADH1B* and *ADH1C* show polymorphisms in Caucasians, for which functional studies have been performed[13,14].

Genotypes of *ADH1B* and *ADH1C* could either directly influence BC risk or modify the association of alcohol and BC. Several case-control studies have investigated the effect of *ADH1B* genotypes on BC

risk without finding an association[15-19]. Effect modification of the alcohol-BC association by the *ADH1B* SNP rs1229984 has been reported[17,20,21], but this variant is rare in Caucasian populations (minor allele frequency <5%). Case-control and nested case-control studies have also investigated the effect of the two well-characterized SNPs rs698 and rs1693482 in *ADH1C* on BC risk as well as putative effect modification of the established alcohol-BC association[15,18-20,22-25]. The SNPs rs698 and rs1693482 are in complete linkage disequilibrium ($r^2=1$). A recent meta-analysis showed no direct effect on BC risk of these SNPs[26]. Effect modification by rs698 and rs1693482 has been reported in two studies[15,23], but with conflicting results. Another meta-analysis[27] of four studies[19,22,23,25] reported increased risks for drinkers versus non-drinkers in fast metabolizers only (rs698 (TT)).

This study used data from the Netherlands Cohort Study on Diet and Cancer (NLCS), in which a previous analysis after 3.3 years of follow-up has already shown an association of alcohol with postmenopausal BC risk[28]. We investigated the association between alcohol and postmenopausal BC risk after 20.3 years of follow-up and potential effect modification by genetic variability in *ADH1B* and *ADH1C* using a tag SNP approach. In doing so, we also studied genetic variability in *ADH1B* and *ADH1C* in relation to postmenopausal BC directly. A potential role for alcohol in modulating estrogen was further explored by distinguishing between ER and PR status of BC in the analyses.

Materials and Methods

Study population and follow-up

The NLCS[29] is a large prospective cohort study that was initiated in September 1986, among 120 852 Dutch men and women who completed a self-administered questionnaires on diet and other potential cancer risk factors. Approximately 75 percent (90 000 participants) of subjects provided toenail samples for analysis of trace elements in addition to their questionnaires. These toenails were subsequently found to be suitable for genotyping[30,31]. Follow-up of cancer occurrence in the whole cohort was performed through annual record linkage to the Netherlands Cancer Registry and PALGA,

a nationwide database of histo- and cytopathology reports[32]. The full cohort consisted of 62 573 women and 58 279 men aged 55-69 years at baseline. A case-cohort approach has been chosen for reasons of efficiency relating to data processing and analysis[33]. Therefore, a subcohort of 5000 persons (2411 men and 2589 women) was sampled randomly from the full cohort at baseline. Follow-up of the subcohort for migration and vital status in order to estimate the accumulated person-time at risk, was achieved by record linkage with the Municipal population (GBA) registries (>99.9% completeness). Supplementary Figure 1 (<http://carcin.oxfordjournals.org/>) shows numbers of subcohort members and cases with additional information on BC subtypes based on hormone receptor status. After 20.3 years of follow-up, 2438 subcohort members and 3339 cases of postmenopausal breast cancer were available after exclusion of prevalent cancer cases at baseline and cases of non-epithelial and borderline invasive cancer. Further exclusions were due to missing toenail material (767 cases, 456 subcohort members), genotyping call rates <95% (242 cases, 153 subcohort members), incomplete information on exposure (147 cases, 134 subcohort members) and missing values for traditional risk factors for BC (408 cases, 311 subcohort members), which lead to 1775 cases and 1384 subcohort members with complete data for analysis. Further exclusions, for at least one not successfully genotyped SNP among the twelve tag SNPs resulted in 1630 cases and 1301 subcohort members. ER status was available for around 60 percent of cases and progesterone receptor (PR) status for around 45 percent, respectively.

Exposure assessment

The self-administered questionnaire was completed at baseline providing information regarding anthropometric characteristics, dietary habits and demographic and lifestyle factors. The 150-item food frequency questionnaire (FFQ) included items on drinking patterns, such as alcohol intake, types of alcoholic beverages as well as stability of drinking as compared to five years before baseline. Six items measured alcohol consumption: 1) beer 2) red wine 3) white wine 4) sherry, vermouth, port, Campari 5) sweet liquor, egg-nog 6) liquor (e.g. gin, brandy, whiskey). Frequency of consumption of

these items as well as average number of glasses consumed per occasion were asked. The total amount of daily alcohol consumption was calculated based on alcohol content of these items. Validity of the measure of alcohol consumption by the FFQ was investigated by comparing the baseline alcohol consumption to dietary records over nine days in a subgroup of the full cohort. Pearson's correlation coefficient for alcohol intake was 0.86[34]. A good reproducibility for alcohol consumption has been demonstrated by repeating the FFQ in random samples of the NLCS annually from 1987 to 1991[35], as well as a general good performance in ranking participants due to their intake of nutrients and alcohol.

SNP-Selection and genotyping

Toenail DNA has been shown to be suitable for analysis of genetic polymorphisms[30,31]. A tag SNP-approach was used to cover as much genetic variability in *ADH1B* and *ADH1C* as possible. Tag SNPs were selected between 5 kilobases up- and downstream of these genes, in order to cover all SNPs with minor allele frequencies of at least 5%. From the HapMap CEU (Utah Residents with Northern and Western European Ancestry) population, 13 SNPs were ascertained. Using the option of aggressive tagging with an r^2 threshold of 0.8, seven tag SNPs were selected to cover 84% of the genetic variation in *ADH1B* and six tag SNPs were selected to cover 96% of the genetic variation in *ADH1C*. The selected SNPs were rs1159918, rs2075633, rs1693439, rs9307239, rs4147536, rs3811802 and rs17033 for *ADH1B*, and rs698, rs1662033, rs3114046, rs4147542, rs283415 and rs4699741 for *ADH1C*.

Genotyping was performed via iPLEXTM assay for the MassARRAY[®] system (Agena Bioscience GmbH, Hamburg, Germany), a method using single-base primer extension and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry[36]. Adherence of SNPs to Hardy-Weinberg Equilibrium was tested in the subcohort and found to be in place, except for rs4699741 ($p = 0.002$). Nevertheless, this deviation from Hardy-Weinberg Equilibrium is unlikely due to genotyping errors, because all SNPs were genotyped at once using a single assay. Therefore, rs4699741 was not excluded from the analysis. Genotyping for rs17033 was not successful as only one

allele was called, and it had to be excluded from the analysis. Therefore, the final number of SNPs is six for each gene. The coverage of genetic variation was 76% in *ADH1B* and 96% in *ADH1C*.

Statistical analysis

Descriptive analysis was done by calculating the distributions of covariates and genotypes across levels of alcohol intake in the subcohort. For the subsequent analysis of main effects of alcohol intake and genotype, as well as the interaction between both, observations with missing data on covariates were excluded list-wise. Cox -regression was performed to estimate hazard rate ratios (HR) for incident postmenopausal BC and corresponding 95% confidence intervals (CI). The proportional hazard assumption was tested using scaled Schoenfeld residuals[37]. Uninformative censoring can be ruled out, as no women from the subcohort were lost to follow-up during the 20.3 years of follow-up. Standard errors were estimated using the robust Huber-White sandwich estimator to account for the additional variance due to sampling from the subcohort.

The analysis of the effect of alcohol on postmenopausal BC was conducted in two different models: age-adjusted and multivariable-adjusted. Alcohol intake was modeled categorically in five categories (non-drinkers, 0.1-<5g/d, 5-<15g/d, 15-<30g/d, >=30g/d) and continuously as per 10g/d. To test for a linear trend across hazard ratios for postmenopausal BC as estimated using the categorical variable, category codings were replaced by the median of alcohol intake in each category and the variable was entered as a continuous variable in the Cox model. For the multivariable-adjusted analysis, a fixed set of known risk factors was included to adjust for potential confounding: age (55-59 years, 60-64 years, 65-69 years), history of benign breast disease, family history of BC, age at menarche (<12 years, 13-14 years, 15-16 years, >17 years), parity and age at first child birth (nulliparous, 1-2 children and age <25 years, 1-2 children and age >=25 years, >=3 children and age <25 years, >=3 children and age >=25 years), ever use of contraception, ever use of hormone replacement therapy, age at menopause (<45 years, 45-49 years, 50-54 years, >=55 years), level of education (primary school or lower vocational, secondary or medium vocational, higher vocational or university), smoking status (never, ex-smoker,

current), daily energy intake, height, BMI (<18.5, 18.5-<25, 25-<30, >=30kg/m²), and non-occupational physical activity (<=30min/d, >30-<=60min/d, >60-<=90min/d, >90min/d). Marginal effects of tag SNP genotypes were analyzed in age-adjusted models, as confounding of the SNP-BC association is unlikely. A dominant genetic model was used to model SNPs. Multiplicative interactions between alcohol intake and tag SNPs were tested by using cross-product terms in the statistical model. Continuous modelling of alcohol intake (per 10g/d) was chosen for the interaction analysis to overcome power restrictions due to small numbers in some cells of hormone receptor subtypes. Also, generally, a linear relationship was seen between alcohol intake and postmenopausal BC risk. The test statistic, used for tests of interaction between alcohol intake and genotype, and tests of linear trends between alcohol intake and breast cancer risk, was the Wald test.

All of the above described analyses were also performed in subtypes of postmenopausal BC as defined by hormone receptor status (ER +/- and PR +/-). The statistical analysis was carried out with STATA (version 14, StataCorp LP, College Station, TX, USA). Tests of significance were performed two-sided with p-values less than 0.05 being considered significant. To account for multiple testing when examining putative interactions between the SNPs and alcohol intake, false discovery rate (FDR) Q-Values[38-40] were calculated. FDR Q-Values are an estimate of the expected proportion of false positive findings among the results regarded as significant if the p-value corresponding to the FDR Q-Value is judged significant. The Benjamini-Hochberg procedure was used to calculate FDR Q-Values with the *qqvalue* package in Stata[41] correcting for 12 tests in analysis of overall effects and for 48 tests in ER/PR subtype analysis. A decision level of 0.2 for the FDR was chosen, which means that among the results that were regarded interesting for further investigation, twenty percent could in fact be false positive findings. The decision level of 0.2 has been used for candidate gene studies before[42,43]. CIs are reported without correction for multiple testing.

Sensitivity analysis was done by excluding cases diagnosed in the first two years of follow-up to account for potential protopathic bias, by restricting the analysis to those women for whom the questionnaire

indicated stability of drinking over a five-year period, and by excluding former drinkers from the reference category of non-drinkers.

Ethics

Ethical approval for the NLCS has been given by the Institutional Review Boards of Maastricht University (Maastricht, The Netherlands) and the TNO Nutrition and Food Research Institute (Zeist, The Netherlands).

Results

Descriptive analysis of baseline characteristics in the subcohort

Table 1 presents baseline characteristics of subcohort members according to categories of alcohol intake as well as the proportion of variant allele carriers for every tag SNP in each category of alcohol intake in the 1695 subcohort members for whom genotyping was available. One third (32.6%) of women in the subcohort were non-drinkers. Only 3.5 percent were in the highest category corresponding to ≥ 30 g/d of alcohol intake. Compared to abstainers, women who drank any alcohol were more likely to be nulliparous, to have ever used oral contraceptive and hormone replacement therapy, to be leaner, to be smokers, to have a higher level of education and a higher daily energy intake. Furthermore, drinkers had a smaller proportion of a positive family history of BC.

The distribution of variant allele carriers across levels of alcohol intake was rather uniform, except for *ADH1B* rs3811802, with a somewhat higher proportion of variant allele carriers in the alcohol intake category of ≥ 30 g/d and *ADH1C* rs4147542, which showed higher proportions of variant allele carriers for alcohol intake levels of 5- <15 g/d and ≥ 30 g/d.

Association of alcohol and BC

Table 2 shows the results of multivariable-adjusted analysis of alcohol and postmenopausal BC risk overall and by hormone receptor (ER/ PR) status. After multiple exclusion processes (Supplementary Figure 1, <http://carcin.oxfordjournals.org/>), 1775 incident cases and 1384 subcohort members contributing 24 275 person-years were available during 20.3 years of follow-up. Alcohol consumption showed a statistically significant positive trend with overall postmenopausal BC risk ($p_{\text{trend}}=0.031$ in multivariable-adjusted analysis). Compared to abstainers, the HR for overall BC was 1.69 (95% CI: 1.10-2.60) for alcohol consumption of $\geq 30\text{g/d}$, while HRs were non-significant for lower categories of alcohol intake. Continuous modelling of alcohol intake per 10 g/d yielded a HR for overall BC of 1.09 (95% CI: 1.01 – 1.19), and the HR for ER+ breast tumors was similar, i.e. 1.09 (95% CI: 0.99 – 1.20). HRs for ER- and PR+ breast tumors were 1.04 (95% CI: 0.87 – 1.25) and 1.05 (95% CI: 0.93 – 1.18) when modelling alcohol intake per 10g/d; for PR- breast tumors the HR was 1.16 (95% CI: 1.00 - 1.34). The results for the age-adjusted model were similar in magnitude and direction to the multivariable-adjusted model (data not shown). Results differed somewhat in their magnitude but not direction when restricting the analysis to those women for whom complete genotyping data was available (1630 cases, 22 841 person-years) (data not shown).

Associations of tag SNP genotype and postmenopausal BC

Table 3 displays the associations between variant allele carriers and overall postmenopausal BC for each tag SNP in *ADH1B* and *ADH1C*. All point estimates were close to one and none reached significance. Results for the analysis of associations of tag SNPs with the risk of hormonal receptor subtypes of postmenopausal BC did not show any systematic differences among receptor subtypes as compared to overall BC. For rs2075633, HRs for variant allele carriers (TC/ CC) as compared to wild type homozygotes (TT) were below one but the HR was not statistically significantly decreased for overall BC, while HRs were statistically significantly decreased for all hormone receptor subgroups, indicating an inverse association (data not shown).

Interaction analysis of alcohol intake and tag SNP genotype

Results of the interaction analysis of alcohol intake as per 10g/d with tag SNPs in relation to overall postmenopausal BC risk are presented in Table 4. Associations of alcohol intake with overall postmenopausal BC were not significantly different between wild type and variant allele carriers as indicated by the P for interaction after correction for multiple testing. Effect modification of the alcohol-BC association by rs1159918 was closest to significance ($p_{\text{interaction}}=0.018$, $q_{\text{interaction}}=0.216$). Within variant allele carriers (AC/ AA) for this tag SNP, a HR for BC of 1.18 (95% CI: 1.07 – 1.31) was observed per 10g/d of alcohol intake, whereas within wild type homozygotes (CC), the HR for BC was 0.98 (95% CI: 0.86 – 1.11) per 10g/d of alcohol intake. For rs4147536, effect modification was second closest to significance. Within variant allele carriers (CA/ AA), a HR for BC of 1.20 (95% CI: 1.06 – 1.35) was observed per 10g/d of alcohol intake, while for wild type homozygotes (CC) the HR was 1.03 (95% CI: 0.93 – 1.14) per 10g/d ($p_{\text{interaction}}=0.051$, $q_{\text{interaction}}=0.306$).

Table 5 shows the results for the interaction analysis applying a continuous model of alcohol intake, specified by hormone receptor status. Similar patterns as for the analysis of interaction in overall BC were seen in the different tumor subtypes. None of the results for interaction analysis were statistically significant after correction for multiple testing.

Marginal effects of alcohol intake on postmenopausal breast cancer and interaction analysis using categories of alcohol intake

Modelling alcohol intake in three categories (non-drinkers, 0.1-<30g/d, >=30g/d) yielded HRs of 1.10 (95% CI: 0.93-1.31) for drinkers of 0.1-<30g/d and 1.69 (95% CI: 1.10-2.59) for drinkers of >=30g/d compared to abstainers for overall postmenopausal BC (data not shown). Results for the interaction analysis between alcohol intake and tag SNPs using a dominant genetic model showed similar patterns of alcohol associated with overall postmenopausal BC risk in wild type homozygotes and in variant allele carriers. Generally, point estimates increased with increasing alcohol intake (data not shown). No statistically significant associations or interactions remained after correction for multiple testing.

Regarding hormonal receptor subtypes of tumors, no significant interaction between alcohol intake and *ADH1B* and *ADH1C* genotype was observed after correction for multiple testing (data not shown). For ER+ and PR- breast tumors, risk estimates increased or remained constant with increasing alcohol intake in variant allele carriers as well as in wild type homozygotes. For ER- and PR+ breast tumors, patterns across different tag SNPs were not as uniform. In general, risk estimates increased with increasing alcohol intake in wild type homozygotes as well as in variant allele carriers. However, interactions between alcohol intake and the tag SNPs investigated were not statistically significant after correction for multiple testing. No tag SNP was shown to consistently modify postmenopausal BC risk due to alcohol intake in a continuous as well as a categorical model of alcohol intake.

Sensitivity Analysis

A sensitivity analysis for protopathic bias was done by exclusion of the first two years of follow-up. The general pattern observed for the alcohol-associated risk of postmenopausal BC did not change overall or for subgroups of disease defined by hormone receptor status after exclusion of the first two years of follow-up. (data not shown).

Restricting the analysis to women who reported stable drinking behavior as compared to five years before baseline resulted in a somewhat weaker association of alcohol intake with postmenopausal BC. Directions of risk estimates for postmenopausal BC were similar for the continuous modelling of alcohol intake, whereas risk estimates for the categorical modelling of alcohol intake showed reversed directions for some of the risk estimates which, nevertheless, stayed close to one (data not shown)

Results for the association of alcohol with postmenopausal BC risk after exclusion of former drinkers from the category of non-drinkers, which separates the abstainers from former-drinkers in the reference category, are shown in table 6. There was no change in the direction of the risk estimates. Magnitudes were only mildly changed.

Discussion

While alcohol intake was shown to be associated with postmenopausal BC risk in this large population-based cohort study, tag SNPs in *ADH1B* and *ADH1C* were not significantly associated with postmenopausal BC risk. No significant effect modification of the well-established alcohol-BC association by tag SNPs was seen in overall BC or by ER or PR subtype.

Ethanol is thought to potentially cause BC through several mechanisms: by its main and cancerous metabolite acetaldehyde, by production of free radicals, by influencing levels of estrogen, as well as by interference with one carbon metabolism[7]. Ethanol and acetaldehyde are both classified as group 1 carcinogens by the IARC[44], meaning that there is sufficient evidence of carcinogenicity in humans. In the absence of *ALDH* alleles that cause slow oxidation of acetaldehyde, the levels of acetaldehyde produced in the liver after ingestion of alcohol depend on the kinetic property of ADH. Mutations associated with fast metabolism by this enzyme will therefore result in higher systemic acetaldehyde levels that could potentially reach and affect breast tissue. This mechanism has been described as a likely pathway for the association of alcohol and cancers such as head and neck, esophageal, colorectal, liver, and stomach cancer[45,46]. On the other hand, slow oxidation of ethanol to acetaldehyde results in longer systemic circulation of ethanol. Additionally, ethanol influences levels of estrogen which plays a role, at least in ER+ BC. Ethanol intake has been shown to elevate estrogen levels in premenopausal as well as postmenopausal women, with effects being more pronounced in a premenopausal state[6]. Fast metabolizers will clear ethanol faster, which could therefore decrease the BC risk associated with this mechanism. As these potential mechanisms of pathogenesis, i.e. the elevated levels of acetaldehyde on the one hand and the elevated levels of estrogens on the other hand, depend on opposed properties of ADH (fast/ slow metabolizer), but might still both be involved in the progression of BC disease, only large changes in kinetic properties are likely to contribute to a relevant change in BC risk. For example, for *ADH1B* rs1229984, the increase in velocity of ethanol turn-over is almost by a factor ninety in variant allele homozygotes as compared to wild type homozygotes, whereas for

ADH1C rs698, the velocity of turn-over is around half the size in variant allele homozygotes as compared to wild type homozygotes[14].

In the cohort under study, main effects of alcohol on overall as well as ER- and PR-specified postmenopausal BC were in line with previous reports[9,10]. Non-significance of results in subtypes of tumors by receptor status are most likely explainable by relatively small sample sizes in the hormone receptor strata. Generally, risk estimates for ER+ tumors aligned best with overall postmenopausal BC risk, which reflects the high proportion of ER+ tumors among all cases. As we did not see substantial differences in alcohol-associated risk estimates between hormone receptor subtypes, our results render the potential involvement of estrogen in alcohol-associated BC less likely. Several studies investigated associations of functional SNPs in *ADH1B*[15-21] and *ADH1C*[15,18-20,22-24,26,27] with BC risk. All of these studies used a candidate SNP approach. For *ADH1B*, most published research has focused on rs1229984, a functional SNP largely absent in Caucasian populations and therefore not included in our analysis. No evidence, so far, is available for direct effects on BC risk. Nevertheless, effect modification of the alcohol-BC association by this SNP was found in three case-control studies[17,20,21]. A direct effect of *ADH1C* genotype (rs698) on BC risk has been reported by two studies[24,25], but was not confirmed in a more recent meta-analysis[26]. This aligns well with the absence of a direct effect of rs698 genotype on BC risk in our study. Effect modification of the alcohol-BC association by *ADH1C* genotypes was shown by Terry et al[23] and Benzon et al[15] with conflicting directions. BC-risk was elevated for slow/intermediate metabolizers (rs698 CC/ CT) in a continuous model of alcohol intake[15] with a RR of 1.14 (95% CI: 1.04 – 1.24) per 10g/d, whereas fast metabolizers (rs698 TT) showed a RR of 0.99 (95% CI: 0.89 – 1.11). In contrast, Terry et al[23] reported an OR of 2.00 (95% CI: 1.10 – 3.50) for fast metabolizers (rs698 TT) who drank 15 to 30 g/d compared to abstainers, an OR of 1.50 (95% CI: 0.90 – 2.40) for intermediate (rs698 CT) and 1.30 (95% CI: 0.50 – 3.50) for slow metabolizers (rs698 CC) in a categorical model of lifetime alcohol intake[23]. In our study, the HR for postmenopausal BC was of comparable size, i.e. 1.13 (95% CI: 1.02 – 1.24) per 10g/d of alcohol intake

in rs698 variant allele carriers (rs698 CC/ CT), as the one reported by Benzou et al[15]. Nevertheless, effect modification was not present.

We used a tag SNP approach to cover as much genetic variability in *ADH1B* and *ADH1C* as possible. As no main effects of tag SNP genotype were seen, the lack of effect modification of the alcohol BC association by tag SNP genotype is in line with expectations. Tag SNPs usually only show minimal risk associations, possibly due to imperfect correlations with represented SNPs and/or additional gene-gene interactions that are not depicted by the tag SNP approach[47].

So far, no approach has been undertaken to comprehensively investigate the potential effect modification by covering a large genetic variability in *ADH1B* and *ADH1C*. With a considerably high coverage of 76% of common genetic variability in *ADH1B* and 96% in *ADH1C* our results do not support an important role of common *ADH1B* and *ADH1C* variations in breast cancer development, neither in women who drink, nor in abstainers.

Strengths of our study are its prospective design, the large number of participants, its population-based approach and the almost complete and long (20.3 years) follow-up through data linkage with cancer registries. Information on alcohol intake was recorded by a well validated FFQ which was shown to rank participants well[34,35].

Our study also has limitations. Self-reported intake of alcohol can cause non-differential misclassification due to social desirability and attenuate risk estimates. This attenuation of the regression coefficient might be greater using a continuous model of alcohol intake than with a categorical model of alcohol intake. Nevertheless, women in our study were between 55 and 69 years at baseline and showed a stable dietary intake[35]. Alcohol intake in particular correlated well with dietary records in a validation study[34]. Ascertainment of hormone receptor status was done in different laboratories over many years which can result in nondifferential misclassification due to differing techniques and protocols. Hormone receptor status was only available for around 60 percent

of cases, which reduced the power of subtype analyses. Finally, the cut-off value chosen for the FDR Q-values of 0.2 is arbitrary, however it has been used in candidate gene studies before[42,43].

In conclusion, in this large prospective cohort study, alcohol intake was associated with overall postmenopausal BC risk as well as with subtypes defined by ER and PR status. No significant effect modification of this association by *ADH1B* and *AHD1C* variability was observed.

Supplementary Material

Supplementary Figure 1 can be found at <http://carcin.oxfordjournals.org/>

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Table 1 Distribution of baseline characteristics and genotype of subcohort members^a according to levels of alcohol intake. Netherlands Cohort Study, 1986-2006.

	Average daily alcohol intake (g/day)				
	0	0.1-<5	5-<15	15-<30	>=30
Demographic and lifestyle					
Characteristics of subcohort members:					
Participants, N (%)	552 (32.6)	612 (36.1)	317 (18.7)	154 (9.1)	60 (3.5)
Age [years], mean (SD)	61.7 (4.3)	61.4 (4.3)	61.3 (4.2)	61.0 (4.5)	60.4 (3.5)
Age at menarche [years], mean (SD)	13.6 (1.8)	13.8 (1.8)	13.8 (1.8)	13.5 (1.6)	13.4 (1.5)
Age at first birth [years], mean (SD)	26.7 (4.5)	27.1 (4.4)	26.9 (3.7)	26.8 (3.2)	25.8 (3.2)
Nulliparous women, %	17.5	18.7	17.3	22.0	21.7
Age at menopause [years], mean (SD)	48.3 (4.8)	49.0 (4.3)	48.9 (4.6)	49.0 (4.3)	48.9 (4.4)
Ever use of oral contraception, %	21.0	23.5	32.1	34.0	33.3
Ever use of HRT, %	10.3	12.5	14.0	17.1	15.0
Positive family history of breast cancer, %	10.0	9.2	7.9	7.8	6.7
Positive history of benign breast disease, %	6.7	8.0	8.8	5.2	6.7
Height [cm], mean (SD)	164.8 (6.5)	165.2 (6.2)	165.3 (5.8)	166.2 (5.5)	164.7 (6.1)
BMI [kg/m ²], mean (SD)	25.5 (3.8)	25.1 (3.5)	24.5 (3.3)	24.4 (3.3)	24.5 (3)
Current Smoker, %	17.9	16.2	23.0	34.4	45.0
Higher vocational schooling or university, %	4.9	7.7	14.6	13.2	21.7
Daily energy intake [kcal], mean (SD)	1622 (417)	1689 (375)	1732 (379)	1777 (394)	1865 (448)
Non-occupational physical activity <=30min/d, %	28.6	22.9	18.8	21.6	13.6
Genotype and variant alleles^b					
ADH1B					
rs1159918 (AA/AC), %	61.8	59.6	57.7	58.6	61.7
rs1693439 (AA/AG), %	15.0	15.0	18.0	13.6	15.0
rs2075633 (CC/CT), %	46.7	49.6	53.3	53.2	43.3
rs3811802 (GG/GA), %	71.6	71.4	63.4	71.8	83.3
rs4147536 (AA/AC), %	37.1	39.2	33.5	31.4	35.0
rs9307239 (TT/TC), %	58.6	60.9	63.7	59.7	63.3
ADH1C					
rs1662033 (GG/GT), %	51.8	53.9	49.8	48.7	48.3
rs283415 (CC/CT), %	67.0	68.3	63.7	63.0	61.7
rs3114046 (TT/TC), %	15.0	15.0	18.0	13.6	15.0
rs4147542 (CC/CT), %	41.7	48.0	56.5	45.3	50.9
rs4699741 (CC/CT), %	15.4	11.3	13.6	11.7	11.7

rs698	(CC/CT), %	64.5	66.4	60.6	59.7	58.3
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Abbreviations: BMI, body mass index; HRT, hormone replacement therapy, rsID, reference SNP ID number; A, adenine; C, cytosine; G, guanine; T, thymine

^aSubcohort members with missing data on alcohol intake were excluded, whereas no exclusions due to missing data on covariates occurred.

Continuous variables are displayed as mean and standard deviation (SD). Categorical variables are given in column percentages per category of alcohol intake

^bPercentages of variant allele carriers (variant homozygotes and heterozygotes) are given for each tag SNP. The total number of subcohort members for whom toenail-DNA and data on alcohol intake were available, was 1695.

Table 2 Multivariable-adjusted^a HRs and 95% CIs according to alcohol intake for postmenopausal breast cancer risk overall and by hormone receptor status. Netherlands Cohort Study, 1986-2006.

	Total alcohol intake [g/d]					P-Value Test for trend	HR (95% CI) per 10g/d
	Non-drinkers	>0-<5	5-<15	15-<30	>=30		
Personyears in subcohort	7740	8668	4754	2348	766		24275
Total breast cancer							
No. of cases	514	648	343	187	83		1775
HR (95% CI)	1 (Ref)	1.10 (0.92 - 1.33)	1.06 (0.84 - 1.33)	1.19 (0.89 - 1.59)	1.69 (1.10 - 2.60)	0.031	1.09 (1.01 - 1.19)
By ER status							
ER+							
No. of cases/ Py	247	311	164	88	35		845
HR (95% CI)	1 (Ref)	1.10 (0.88 - 1.38)	1.05 (0.80 - 1.38)	1.15 (0.81 - 1.63)	1.51 (0.91 - 2.52)	0.185	1.09 (0.99 - 1.20)
ER-							
No. of cases/ Py	66	68	34	20	9		197
HR (95% CI)	1 (Ref)	0.89 (0.60 - 1.31)	0.78 (0.48 - 1.26)	0.92 (0.51 - 1.65)	1.32 (0.58 - 3.01)	0.694	1.04 (0.87 - 1.25)
By PR status							
PR+							
No. of cases/ Py	153	184	96	51	18		502
HR (95% CI)	1 (Ref)	1.03 (0.78 - 1.34)	0.96 (0.69 - 1.34)	1.02 (0.67 - 1.55)	1.23 (0.67 - 2.24)	0.684	1.05 (0.93 - 1.18)
PR-							
No. of cases/ Py	83	107	53	31	17		291
HR (95% CI)	1 (Ref)	1.13 (0.81 - 1.57)	0.97 (0.64 - 1.48)	1.19 (0.72 - 1.98)	1.87 (0.95 - 3.68)	0.147	1.16 (1.00 - 1.34)

Abbreviations: HR, hazard rate ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor

^aAll multivariable-adjusted models included the following confounders: age (55-59, 60-64, 65-69), positive history of benign breast disease, positive family history of breast cancer, age at menarche (<12, 13-14, 15-16,>17), compound variable of parity and age at first birth (nulliparous, 1-2 children and age <25, 1-2 children and age ≥25, ≥3 children and age <25, ≥3 children and age ≥25), ever use of contraception, ever use of HRT, age at menopause (<45, 45-49, 50-54, ≥55), level of education (primary school or lower vocational, secondary or medium vocational, higher vocational or university), smoking status(never/ex-smoker/current), daily energy intake, height, BMI (<18.5, 18.5-<25, 25-<30, ≥30kg/m²), non-occupational physical activity (<=30min/d, >30-<=60min/d, >60-<=90min/d, >90min/d)

Table 3 HR and 95% CI for postmenopausal breast cancer risk according to alcohol dehydrogenase genetic variants using a dominant genetic model in women with available toe nail material. Netherlands Cohort Study, 1986-2006.

SNP	No. cases	Person-years	HR	95% CI	p Value	FDR Q-Value
ADH1B						
rs1159918						
Homozygote wild type (CC)	938	12828	1 (Ref)			
Variant allele carrier (AC/AA)	1390	19029	1.00	0.88 - 1.14	0.993	0.993
rs1693439						
Homozygote wild type (GG)	2006	26957	1 (Ref)			
Variant allele carrier (GA/AA)	324	4960	0.88	0.74 - 1.04	0.142	0.415
rs2075633						
Homozygote wild type (TT)	1230	16012	1 (Ref)			
Variant allele carrier (TC/CC)	1099	15905	0.90	0.79 - 1.01	0.083	0.415
rs3811802						
Homozygote wild type (AA)	674	9536	1 (Ref)			
Variant allele carrier (AG/GG)	1655	22369	1.05	0.91 - 1.20	0.517	0.620
rs4147536						
Homozygote wild type (CC)	1422	20044	1 (Ref)			
Variant allele carrier (CA/AA)	908	11860	1.08	0.95 - 1.23	0.227	0.454
rs9307239						
Homozygote wild type (CC)	856	12537	1 (Ref)			
Variant allele carrier (CT/TT)	1472	19363	1.12	0.98 - 1.27	0.087	0.415
ADH1C						
rs1662033						
Homozygote wild type (TT)	1101	15493	1 (Ref)			
Variant allele carrier (TG/GG)	1228	16425	1.05	0.93 - 1.19	0.400	0.533
rs283415						
Homozygote wild type (TT)	749	10749	1 (Ref)			
Variant allele carrier (TC/CC)	1580	21189	1.07	0.94 - 1.22	0.298	0.469
rs3114046						
Homozygote wild type (CC)	2002	26957	1 (Ref)			
Variant allele carrier (CT/TT)	328	4980	0.89	0.74 - 1.05	0.173	0.415
rs4147542						
Homozygote wild type (TT)	1125	15856	1 (Ref)			
Variant allele carrier (TC/CC)	1023	14305	1.01	0.89 - 1.15	0.893	0.974
rs4699741						
Homozygote wild type (TT)	2066	27792	1 (Ref)			
Variant allele carrier (TC/CC)	264	4145	0.86	0.71 - 1.04	0.113	0.415
rs698						
Homozygote wild type (TT)	808	11553	1 (Ref)			
Variant allele carrier (TC/CC)	1520	20368	1.07	0.94 - 1.22	0.313	0.469

Abbreviations: rsID, reference SNP ID number; A, adenine; C, cytosine; G, guanine; T, thymine; CI, confidence interval; FDR, False Discovery Rate

Results are age-adjusted and displayed as hazard ratios (HR) for variant allele carriers (heterozygotes and variant allele homozygotes versus wild type homozygotes). FDR Q-Values are calculated as described in the methods section with correction for 12 tests. A threshold of 0.2 would be considered significant.

Table 4 HRs and 95% CIs for postmenopausal breast cancer risk according to alcohol intake (continuous per 10g/d) and genotype of Tag SNPs in ADH1B and ADH1C in women with available toe nail material. Netherlands Cohort Study, 1986-2006^a.

	No. cases	Person-years	Per 10g/d		P-value Interaction	FDR Q-Value ^b
			HR	95% CI		
Alcohol effects in full sample	1775	24276	1.09	1.01 – 1.19		
Tag SNP						
ADH1B						
rs1159918						
Homozygote wild type (CC)	725	9603	0.98	0.86 – 1.11		
Variant allele carrier (AC/AA)	1048	14609	1.18	1.07 – 1.31	0.018	0.216
rs1693439						
Homozygote wild type (GG)	1525	20494	1.10	1.01 – 1.20		
Variant allele carrier (GA/AA)	250	3781	1.08	0.89 – 1.30	0.844	0.875
rs2075633						
Homozygote wild type (TT)	937	12029	1.12	0.74 – 1.07		
Variant allele carrier (TC/CC)	837	12247	1.06	0.94 – 1.19	0.437	0.750
rs3811802						
Homozygote wild type (AA)	511	7207	1.11	0.95 – 1.28		
Variant allele carrier (AG/GG)	1263	17036	1.09	0.99 – 1.19	0.858	0.875
rs4147536						
Homozygote wild type (CC)	1096	15284	1.03	0.93 – 1.14		
Variant allele carrier (CA/AA)	679	8971	1.20	1.06 – 1.35	0.051	0.306
rs9307239						
Homozygote wild type (CC)	682	9122	1.09	0.96 – 1.23		
Variant allele carrier (CT/TT)	1091	15116	1.10	1.00 – 1.22	0.875	0.875
ADH1C						
rs1662033						
Homozygote wild type (TT)	838	11829	1.05	0.94 – 1.18		
Variant allele carrier (TG/GG)	936	12427	1.13	1.01 – 1.26	0.379	0.750
rs283415						
Homozygote wild type (TT)	578	8174	1.05	0.92 – 1.21		
Variant allele carrier (TC/CC)	1196	16101	1.11	1.01 – 1.22	0.492	0.750
rs3114046						
Homozygote wild type (CC)	1521	20494	1.10	1.01 – 1.20		
Variant allele carrier (CT/TT)	254	3781	1.07	0.88 – 1.29	0.779	0.875
rs4147542						
Homozygote wild type (TT)	869	11896	1.13	1.00 – 1.27		
Variant allele carrier (TC/CC)	771	11134	1.05	0.94 – 1.18	0.397	0.750
rs4699741						
Homozygote wild type (TT)	1578	21284	1.08	1.00 – 1.18		
Variant allele carrier (TC/CC)	197	2991	1.19	0.91 – 1.56	0.500	0.750
rs698						
Homozygote wild type (TT)	618	8810	1.02	0.89 – 1.16		
Variant allele carrier (TC/CC)	1155	15448	1.13	1.02 – 1.24	0.203	0.750

Abbreviations: rsID, reference SNP ID number; A, adenine; C, cytosine; G, guanine; T, thymine; FDR, False Discovery Rate
^aAll models were adjusted for the following confounders: age (55-59, 60-64, 65-69), positive history of benign breast disease, positive family history of breast cancer, age at menarche (<12, 13-14, 15-16, >17), compound variable of parity and age at first birth (nulliparous, 1-2 children and age <25, 1-2 children and age ≥25, ≥3 children and age <25, ≥3 children and age ≥25),

ever use of contraception, ever use of HRT, age at menopause (<45, 45-49, 50-54, >=55), level of education (primary school or lower vocational, secondary or medium vocational, higher vocational or university), smoking status(never/ex-smoker/current), daily energy intake, height, BMI (<18.5, 18.5-<25, 25-<30, >=30kg/m²), non-occupational physical activity (<=30min/d, >30-<=60min/d, >60-<=90min/d, >90min/d)

^bFDR Q-Values are calculated as described in the methods section with correction for 12 tests. A threshold of 0.2 would be considered significant.

Table 5 HRs and 95% CIs for hormonal receptor subtypes postmenopausal breast cancer risk according to alcohol intake (continuous per 10g/d) and genotype of Tag SNPs in ADH1B and ADH1C in women with available toe nail material. Netherlands Cohort Study, 1986-2006^a.

	ER +			ER -			PR +			PR -						
	No. cases	Per 10g/d HR	P-value (95% CI) Interaction ^b	No. cases	Per 10g/d HR	P-value (95% CI) Interaction ^b	No. cases	Per 10g/d HR	P-value (95% CI) Interaction ^b	No. cases	Per 10g/d HR	P-value (95% CI) Interaction ^b				
Alcohol effects in full sample	845	1.09	0.99 – 1.20	197	1.04	0.87 – 1.25	502	1.05	0.93 – 1.18	291	1.16	1.00 – 1.34				
Tag SNP																
ADH1B																
rs1159918																
Homozygote wild type (CC)	333	0.99	0.87 – 1.15	91	0.87	0.61 – 1.23	192	0.96	0.79 – 1.16	132	1.08	0.90 – 1.34				
Variant allele carrier (AC/AA)	511	1.17	1.02 – 1.33	0.099	106	1.19	0.97 – 1.46	0.118	309	1.12	0.96 – 1.30	0.210	159	1.23	1.01 – 1.49	0.365
rs1693439																
Homozygote wild type (GG)	725	1.07	0.96 – 1.19	159	1.04	0.85 – 1.27	429	1.02	0.90 – 1.16	245	1.14	0.98 – 1.34				
Variant allele carrier (GA/AA)	120	1.20	0.95 – 1.52	0.369	38	1.05	0.69 – 1.59	0.972	73	1.22	0.92 – 1.61	0.243	46	1.21	0.84 – 1.74	0.771
rs2075633																
Homozygote wild type (TT)	459	1.12	0.99 – 1.27	96	1.06	0.84 – 1.33	269	1.04	0.90 – 1.21	158	1.19	0.98 – 1.44				
Variant allele carrier (TC/CC)	386	1.05	0.91 – 1.22	0.519	100	1.04	0.79 – 1.37	0.923	233	1.06	0.88 – 1.27	0.884	132	1.12	0.90 – 1.39	0.694
rs3811802																
Homozygote wild type (AA)	231	1.10	0.91 – 1.33	64	0.94	0.64 – 1.38	148	1.09	0.90 – 1.32	79	1.18	0.84 – 1.65				
Variant allele carrier (AG/GG)	614	1.09	0.97 – 1.21	0.888	133	1.08	0.88 – 1.32	0.527	354	1.03	0.90 – 1.19	0.668	212	1.15	0.98 – 1.35	0.885
rs4147536																
Homozygote wild type (CC)	515	1.03	0.91 – 1.71	134	1.01	0.80 – 1.29	300	1.00	0.79 – 1.37	205	1.14	0.95 – 1.37				
Variant allele carrier (CA/AA)	330	1.19	1.02 – 1.38	0.142	63	1.11	0.85 – 1.44	0.609	202	1.13	0.95 – 1.36	0.268	86	1.19	0.93 – 1.52	0.792
rs9307239																

Homozygote wild type (CC)	297	1.11	0.95 – 1.30		89	1.07	0.83 – 1.39		176	1.09	0.91 – 1.31		124	1.16	0.93 – 1.45	
Variant allele carrier (CT/TT)	548	1.08	0.95 – 1.22	0.729	106	1.03	0.80 – 1.34	0.836	326	1.02	0.88 – 1.19	0.590	165	1.16	0.95 – 1.41	1.000
ADH1C																
rs1662033																
Homozygote wild type (TT)	412	1.06	0.92 – 1.22		93	0.98	0.74 – 1.29		231	1.02	0.85 – 1.22		146	1.13	0.91 – 1.39	
Variant allele carrier (TG/GG)	432	1.12	0.98 – 1.28	0.549	104	1.10	0.87 – 1.38	0.526	270	1.08	0.93 – 1.26	0.618	145	1.18	0.97 – 1.44	0.745
rs283415																
Homozygote wild type (TT)	272	1.02	0.85 – 1.22		69	0.94	0.68 – 1.28		151	0.94	0.75 – 1.19		107	1.15	0.89 – 1.48	
Variant allele carrier (TC/CC)	572	1.12	1.00 – 1.26	0.348	128	1.09	0.88 – 1.35	0.423	351	1.10	0.96 – 1.25	0.256	184	1.16	0.98 – 1.39	0.923
rs3114046																
Homozygote wild type (CC)	722	1.07	0.97 – 1.19		159	1.04	0.85 – 1.27		426	1.02	0.90 – 1.16		245	1.14	0.98 – 1.34	
Variant allele carrier (CT/TT)	123	1.19	0.94 – 1.50	0.425	38	1.05	0.69 – 1.59	0.972	76	1.20	0.90 – 1.59	0.310	46	1.21	0.84 – 1.74	0.771
rs4147542																
Homozygote wild type (TT)	400	1.13	0.98 – 1.30		107	1.09	0.87 – 1.38		226	1.10	0.93 – 1.29		149	1.18	0.97 – 1.44	
Variant allele carrier (TC/CC)	382	1.07	0.93 – 1.24	0.610	82	0.99	0.74 – 1.34	0.616	234	1.02	0.85 – 1.22	0.532	127	1.18	0.94 – 1.47	0.983
rs4699741																
Homozygote wild type (TT)	745	1.08	0.97 – 1.20		174	1.03	0.85 – 1.26		431	1.05	0.92 – 1.19		253	1.14	0.98 – 1.34	
Variant allele carrier (TC/CC)	100	1.20	0.86 – 1.68	0.551	23	1.15	0.69 – 1.91	0.710	71	1.14	0.81 – 1.60	0.634	38	1.35	0.83 – 2.20	0.525
rs698																
Homozygote wild type (TT)	289	1.01	0.85 – 1.20		76	0.88	0.64 – 1.21		161	0.96	0.77 – 1.19		115	1.09	0.84 – 1.41	
Variant allele carrier (TC/CC)	555	1.13	1.01 – 1.27	0.274	121	1.12	0.91 – 1.38	0.206	341	1.10	0.96 – 1.26	0.282	176	1.19	1.00 – 1.41	0.560

Abbreviations: rsID, reference SNP ID number; A, adenine; C, cytosine; G, guanine; T, thymine

^aAll models were adjusted for the following confounders: age (55-59, 60-64, 65-69), positive history of benign breast disease, positive family history of breast cancer, age at menarche (<12, 13-14, 15-16,>17), compound variable of parity and age at first birth (nulliparous, 1-2 children and age <25, 1-2 children and age ≥25, ≥3 children and age <25, ≥3 children and age ≥25), ever use of contraception, ever use of HRT, age at menopause (<45, 45-49, 50-54, ≥55), level of education (primary school or lower vocational, secondary or medium vocational, higher vocational or university), smoking status(never/ex-smoker/current), daily energy intake, height, BMI (<18.5, 18.5-<25, 25-<30, ≥30kg/m²), non-occupational physical activity (<=30min/d, >30-<=60min/d, >60-<=90min/d, >90min/d)

^bP-values are shown before correction for multiple testing. FDR Q-Values have been calculated as described in the methods section. All FDR Q-Values were above 0.2.

Table 6 Sensitivity Analysis for exclusion of former drinkers from the reference category. Multivariable-adjusted^a HRs and 95% CIs for postmenopausal breast cancer risk overall and by hormone receptor status are presented according to alcohol. After exclusion of former drinkers from the category of abstainers, 1596 cases and 22029 person-years were available for analysis. Netherlands Cohort Study, 1986-2006.

	Total alcohol intake [g/d]					P-Value Test for trend	HR (95% CI) per 10g/d
	Non-drinkers	>0-<5	5-<15	15-<30	>=30		
Personyears in subcohort	7454	8668	4754	2348	766		23990
Total breast cancer							
No. of cases	488	648	343	187	83		1749
HR (95% CI)	1 (Ref)	1.13 (0.93-1.36)	1.07 (0.85-1.35)	1.21 (0.90-1.61)	1.72 (1.12-2.64)	0.029	1.10 (1.01 - 1.19)
By ER status							
ER+							
No. of cases/ Py	236	311	164	88	35		834
HR (95% CI)	1 (Ref)	1.11 (0.89-1.40)	1.05 (0.80-1.39)	1.15 (0.81-1.64)	1.52 (0.91-2.52)	0.200	1.09 (0.99 - 1.20)
ER-							
No. of cases/ Py	63	68	34	20	9		194
HR (95% CI)	1 (Ref)	0.90 (0.61-1.34)	0.77 (0.47-1.27)	0.93 (0.51-1.67)	1.33 (0.58-3.04)	0.692	1.05 (0.87 - 1.26)
By PR status							
PR+							
No. of cases/ Py	145	184	96	51	18		494
HR (95% CI)	1 (Ref)	1.05 (0.80-1.38)	0.97 (0.69-1.35)	1.03 (0.67-1.57)	1.24 (0.68-2.26)	0.701	1.05 (0.93 - 1.18)
PR-							
No. of cases/ Py	79	107	53	31	17		287
HR (95% CI)	1 (Ref)	1.14 (0.81-1.60)	0.97 (0.63-1.48)	1.19 (0.72-1.98)	1.89 (0.96-3.72)	0.149	1.16 (1.00 - 1.35)

Abbreviations: HR, hazard rate ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor

^aAll multivariable-adjusted models included the following confounders: age (55-59, 60-64, 65-69), positive history of benign breast disease, positive family history of breast cancer, age at menarche (<12, 13-14, 15-16,>17), compound variable of parity and age at first birth (nulliparous, 1-2 children and age <25, 1-2 children and age ≥25, ≥3 children and age <25, ≥3 children and age ≥25), ever use of contraception, ever use of HRT, age at menopause (<45, 45-49, 50-54, ≥55), level of education (primary school or lower vocational, secondary or medium vocational, higher vocational or university), smoking status(never/ex-smoker/current), daily energy intake, height, BMI (<18.5, 18.5-<25, 25-<30, ≥30kg/m²), non-occupational physical activity (<=30min/d, >30-<=60min/d, >60-<=90min/d, >90min/d)